CANINE DOUBLE-LUNG TRANSPLANTATION WITH CADAVERIC DONORS

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If lungs could be retrieved from cadavers after circulatory arrest, the critical shortage of donors for lung transplantation might be alleviated. To assess gas exchange after transplantation of lungs from cadaveric donors, we performed double-lung transplantation through sequential thoracotomies in 12 dogs. Donors were sacrificed by intravenous pentobarbital injection and then ventilated with 100% oxygen. Lungs were harvested 2 hours ($n = 6$) or 4 hours ($n = 6$) after death and flushed with 2 L modified Euro-Collins solution. Recipients underwent sequential right and left lung transplantation; they were then monitored while under anesthesia for 8 hours, with adjustments of the fraction of inspired oxygen. Nine of 12 recipients survived the 8-hour study period. Four of six dogs with cadaveric lungs retrieved 2 hours after death survived; deaths were from pulmonary embolism at 6 hours and pulmonary edema at 2 hours. Five of six dogs with cadaveric lungs retrieved 4 hours after death survived; one died of hypoxia during implantation of the left lung, while dependent on the right lung graft. Postoperative hemodynamic and gas exchange parameters were similar in both groups. Alveolar-arterial oxygen gradient rose significantly compared with baseline 1 hour after transplantation in both groups (462 ± 60 vs 38 ± 31 mm Hg for 2-hour group, $p < 0.0001$, and 484 ± 63 vs 38 ± 14 mm Hg for 4-hour group, $p < 0.0002$). By 8 hours after operation, the gradients had significantly decreased in both groups (105 ± 37 mm Hg for 2-hour group and 146 ± 53 mm Hg for 4-hour group) and were similar to baseline values. Extravascular lung water also rose significantly 1 hour after transplantation (15.7 ± 2.8 vs 7.9 ± 0.5 ml/kg for 2-hour group, $p < 0.02$, and 16.9 ± 1.2 vs 6.6 ± 0.4 ml/kg for 4-hour group, $p < 0.0001$) and decreased gradually during the 8-hour study period. Donor lungs retrieved at 2 and 4 hours postmortem afford similar recipient outcomes. Improvement in alveolar-arterial oxygen gradient and reduction in extravascular lung water during the study period imply that the ischemia-reperfusion injury induced by this model is reversible. If this approach could be safely introduced to clinical practice, substantially more transplant procedures could be performed. (J Thorac Cardiovasc Surg 1996;112:577-83)

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The shortage of donor lungs suitable for clinical transplantation prompted our laboratory to investigate the transplantation of lungs retrieved from circulation-arrested cadavers.[1-3] In these nonsurvival canine experiments, recipients were observed for an 8-hour period after transplantation while gas exchange and hemodynamic data were monitored. During that 8-hour period, the recipient was rendered dependent on the left single-lung allograft by occlusion of the right main stem bronchus and right pulmonary artery. Fraction of inspired oxygen (Fio_2) was maintained at 0.4. Development of a substantial alveolar-arterial oxygen gradient ($A\text{-}aDo_2$) resulted in hypoxemia in recipients and led to death. Even a minimal injury to the endothelial cell layer resulted

in increased interstitial edema by virtue of increased blood flow because the entire cardiac output was directed to the newly transplanted lung. In essence, a single-lung transplantation model with native lung exclusion is an excellent model for the study of subtle differences in the quality of lung preservation, because this model exaggerates the impact of pulmonary preservation that is less than perfect. Such a model may, however, be too rigorous to address the question of tolerable (and recoverable) ischemic injury to the pulmonary parenchyma.

This study investigated whether a double-lung transplantation model would lead to improved recipient survival during the first 8 hours after transplantation with lungs retrieved from circulation-arrested cadavers. This would allow accumulation of more data on gas exchange function. Recipients of lungs harvested at either 2 or 4 hours postmortem were studied.

Methods

Donor preparation. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). The study was approved by the Institutional Animal Care and Use Committee. Heartworm-free adult mongrel dogs were given intravenous heparin (400 U/kg of heparin sodium) before they were sacrificed, to exclude intrapulmonary thrombosis as a cause of poor graft function. After heparin administration, donors were sacrificed by intravenous administration of 65 mg/kg sodium pentobarbital and were immediately intubated and ventilated with 100% oxygen with a tidal volume of 15 ml/kg, a respiratory rate of 12 breaths/min, and positive end-expiratory pressure of 5 cm H_2O. Cadavers were then secured in the supine position and left at room temperature for either 2 or 4 hours.

Lung harvesting. The same methods employed for human lung harvesting were used for bilateral lung extraction from donor animals.[4] Through a median sternotomy, a 24F catheter was inserted into the main pulmonary artery and the left atrial appendage was incised. No postmortem thrombi were found within the pulmonary artery or left atrium in any donor. The lung block was perfused during a 3- to 4-minute period with 1 L modified Euro-Collins solution stored at 4° C. The composition of the solution was as follows: 7.40 gm/L dibasic potassium phosphate, 0.84 gm/L sodium bicarbonate, 2.04 gm/L monobasic potassium phosphate, 1.12 gm/L potassium chloride, 0.48 gm/L magnesium sulfate, 1.00 gm/L cefazolin, 50 ml/L 50% dextrose in water, and 5000 U/L heparin. The pulmonary arterial infusion pressure was maintained at 25 cm H_2O by adjusting the height of the drip chamber. After completion of the flush, the heart was excised and the double-lung block was harvested; a clamp was placed across the trachea. The specimen was separated into right and left lung blocks and then submerged in Euro-Collins solution and stored at 4° C while the recipient animal was prepared.

Recipient preparation and transplantation. Heartworm-free adult mongrel dogs were anesthetized by intravenous administration of 32 mg/kg sodium pentobarbital. Maintenance anesthesia consisted of 12.5 $mg \cdot kg^{-1} \cdot hr^{-1}$ α-chloralose beginning 2 hours after the initial pentobarbital dose. Recipient animals were ventilated with a tidal volume of 15 ml/kg and positive end-expiratory pressure of 5 cm H_2O, with adjustment of the ventilatory rate to maintain normocapnia. Donors and recipients were weight matched.

Cefazolin sodium (1 gm) was administered to recipient animals after induction of anesthesia. A right femoral artery lung water catheter (Baxter Healthcare Corp., Edwards Division, Irvine, Calif.) was placed before a Swan-Ganz catheter (Baxter Healthcare Corp., Edwards Division) was advanced from the right femoral vein.

Bilateral lung allotransplantation was carried out through sequential thoracotomies. A right thoracotomy was performed first; after transplantation, the right side of the chest was closed. A left thoracotomy was then performed, and the left side of the chest was left open after transplantation. A Millar catheter was inserted through the left atrial appendage after completion of the left lung transplant.

Allotransplantation was performed in a manner similar to that described elsewhere.[1] On the right side, after recipient pneumonectomy, the pericardium was opened and the interatrial groove was developed. The recipient left atrial cuff on the right side excluded the ligated mediastinal lobe vein. This lobe is anatomically part of the right lung, and the mediastinal lobe was incorporated into the donor left atrial cuff on the right side. An everting atrial horizontal mattress anastomosis was performed with 5-0 Prolene suture (Ethicon, Inc., Somerville, N.J.). The bronchus was reapproximated with running 4-0 Prolene suture, and 6-0 Prolene suture was used for the pulmonary artery anastomosis. Before completion of the pulmonary arterial suture line, the lung was vented of air in the usual fashion.[5]

Recipients were maintained in the right lateral decubitus position after completion of the left lung transplant. The Fio_2 was adjusted to achieve an oxygen saturation greater than 90%. The ventilatory rate was adjusted as needed to keep the carbon dioxide tension between 35 and 45 mm Hg. If metabolic acidosis developed, sodium bicarbonate was administered intravenously to maintain the pH between 7.35 and 7.40. Lactated Ringer's solution was used as a maintenance intravenous fluid. Diuretics were not employed. Left atrial pressure was maintained between 5 and 10 mm Hg. Interventions were not performed within 15 minutes of measurements. Normothermia was maintained by means of heating pads.

Measurements. Arterial blood gas samples were drawn hourly from a femoral artery line and measured on an Instrumentation Laboratory model 1304 blood gas analyzer. Blood oxygen saturation was measured with an Instrumentation Laboratory model 482 Co-Oximeter (In-

strumentation Laboratory Company, Lexington, Mass.), calibrated from a canine oxyhemoglobin saturation curve. A-aDo_2 ($PAo_2 - Pao_2$) was calculated by approximating as follows:[6] $PAo_2 = PIo_2 - (PACo_2/R)$, where PAo_2 is alveolar oxygen tension, PIo_2 is inspired air oxygen tension, $PAco_2$ is alveolar carbon dioxide tension, and R, the respiratory quotient, is assumed to be 0.8.

Hemodynamic recordings of pressure in the right atrium, pulmonary artery, and aorta were taken every hour for the 8-hour follow-up period. Total pulmonary vascular resistance was calculated according to the following formula: TPR = (PA − PAD)/CO, where *TPR* is total pulmonary resistance, *PA* is mean pulmonary arterial pressure, *PAD* is diastolic pulmonary arterial pressure, and *CO* is cardiac output.

Transducer-derived pressures were recorded with a BNC–34-pin ribbon cable interface on an Apple Macintosh II microcomputer (Apple Computer, Inc., Cupertino, Calif.). In addition, an Edwards Model 9310 Lung Water Computer (Baxter Healthcare Corp., Edwards Division) was used to measure the extravascular lung water by a thermodilution method. All animals were followed up for 8 hours or until death. Animals that survived to the end of the study period were sacrificed by intravenous injection of saturated potassium chloride solution.

Definitions. *Transplantation time* is defined as the time from removal of the lung from storage solution until restoration of blood flow to the organ. *Cold ischemic time* is defined as the time from the initiation of the flush of the donor lung until the lung was removed from cold storage for transplantation. *Total ischemic time* is defined as the sum of transplantation and cold ischemic times, plus 2 or 4 hours depending on study group. Preservation time is defined as the time from flushing the donor lungs to reperfusion in the recipient. Baseline measurements were made in intact recipients before either lung was transplanted.

Statistical analysis. Results are presented as mean (± standard error of the mean). Two-way analysis of variance and unpaired t tests were used to analyze differences between the two study groups. Statistical significance was defined at a p value less than 0.05.

Results

Harvest, transplantation, and preservation times for each lung and each group are presented in Table I. There were no significant differences between the two groups. The ischemic time was almost 2 hours longer on the left side, reflecting the additional ischemic time required for left pneumonectomy and left lung transplantation.

Nine of the 12 dogs survived the 8-hour observation period (Fig. 1). Two dogs in the 2-hour group died before completion of this period, one of hypoxia from pulmonary edema only 1 hour after completion of the second transplant and the other suddenly and unexpectedly 6 hours after transplant. Necropsy demonstrated pulmonary emboli, with thrombus around the Swan-Ganz catheter in the second dog.

Table I. *Comparison of experimental variables*

	Cadaver time	
	2 hr	*4 hr*
Number of dogs	6	6
Donor weight (kg)	23 ± 1	23 ± 1
Recipient weight (kg)	23 ± 1	24 ± 1
Harvest time (min)	21 ± 2	25 ± 4
Transplant times (min)		
Total	126 ± 5	131 ± 8
Right lung	59 ± 2	66 ± 9
Left lung	67 ± 5	63 ± 3
Preservation time (min)		
Right	179 ± 12	183 ± 11
Left	306 ± 12	285 ± 4

One dog in the 4-hour group died of hypoxia during the left lung transplant, while dependent on the right lung allograft. The remaining five recipients of 4-hour cadaveric lungs survived the 8-hour observation period.

Pulmonary vascular resistance increased significantly after transplantation compared with pretransplantation (baseline) values (Fig. 2). This increase, which was approximately 50%, was essentially sustained throughout the study period in both groups.

A-aDo_2 increased dramatically after transplantation in all animals (Fig. 3), from a mean of 37 mm Hg at baseline in both groups of cadaveric recipients to 462 mm Hg in the 2-hour group and 484 mm Hg in the 4-hour group (p not significant between groups). Nevertheless, A-aDo_2 subsequently *decreased* substantially in all animals during the remainder of the study period, excluding the lone animal that died of hypoxia. Eight hours after transplantation, the mean A-aDo_2 was 105 mm Hg in the 2-hour group and 146 mm Hg in the 4-hour group (p not significant between groups). These values differ significantly from A-aDo_2 at 1 hour after transplantation.

Extravascular lung water was also increased in recipients, essentially doubling with respect to baseline and then slowly decreasing with time (Fig. 4). Interestingly, there was a significant correlation between mean extravascular lung water at each interval and mean A-aDo_2 at the same interval in both groups of recipients (Fig. 5). As extravascular lung water decreased after the first hour after transplantation, A-aDo_2 improved.

Discussion

This study demonstrates that lungs retrieved from ventilated canine cadavers can function well and

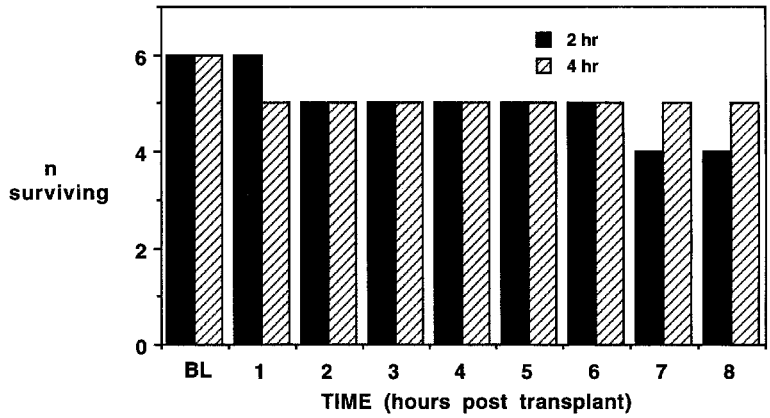

Fig. 1. Number of animals in 2-hour and 4-hour groups surviving after completion of the second transplant.

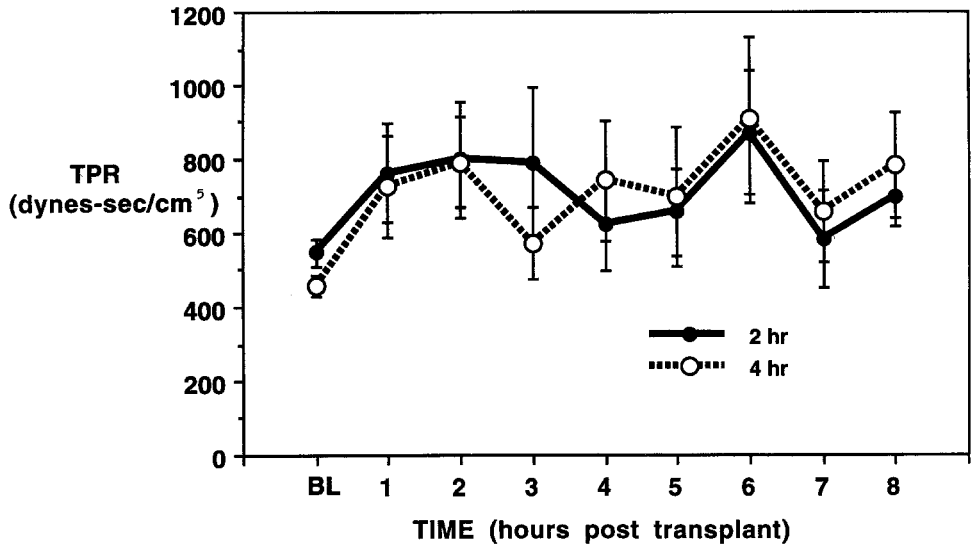

Fig. 2. Total pulmonary resistance *(TPR)* of animals in 2-hour and 4-hour groups (mean ± standard error of the mean).

sustain life, even if retrieved 4 hours after circulatory arrest and death of the donor. The increased pulmonary vascular resistance and extravascular lung water we documented are consistent with a reperfusion injury after transplantation. Reduction in extravascular lung water after the first hour implies that the injury was not ongoing; furthermore, improvement in A-aDo_2 after transplantation is consistent with recovery of lung function. The observed degrees of A-aDo_2 in the last hours of this experiment are commonly seen after clinical lung transplantation and provide additional support for the notion that the lung injury seen in this model is probably recoverable during a short time span.

We believe that this model offers advantages with respect to the single-lung transplant model we initially employed to investigate the hypothesis that lungs retrieved from cadavers could be successfully transplanted.[1] By ligating the opposite (nontransplanted) lung and maintaining recipient Fio_2 at 0.4, subtle reperfusion injuries may have been exaggerated. Development of substantial A-aDo_2, even transiently, was incompatible with survival during the observation period. The single-lung model exag-

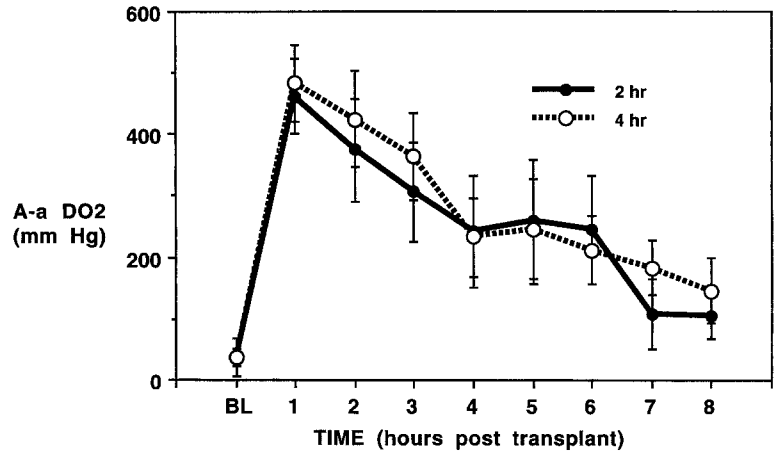

Fig. 3. A-aDo_2 of animals in 2-hour and 4-hour groups (mean ± standard error of the mean).

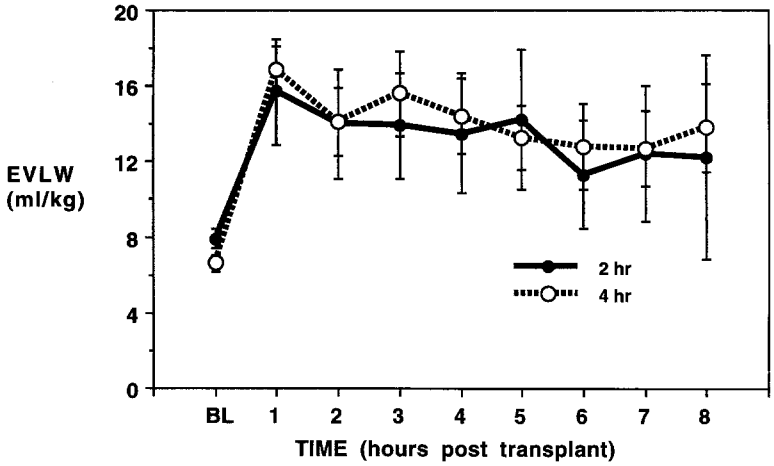

Fig. 4. Extravascular lung water *(EVLW)* of animals in 2-hour and 4-hour groups (mean ± standard error of the mean).

gerates reperfusion injury by placing an unfair burden on the newly transplanted lung, forcing it to accept the entire recipient cardiac output for the duration of the experiment. Although this may be an appropriate strategy if the aim of the study is to evaluate interventions designed to reduce reperfusion injury,[7, 8] it may be a less than optimal method to study the degree of reperfusion injury that is survivable or reversible. The current model requires the recipient to survive with a right lung graft alone only while the left lung is being explanted and transplanted, which takes about 2 hours. During this interval, one animal died of hypoxia. This death might have been prevented in the clinical setting by the institution of cardiopulmonary bypass, which was not available in this study. By treating the recipient animals with increased Fio_2 and positive end-expiratory pressure to address reduced A-aDo_2, we allowed for reversal of transient pulmonary dysfunction and demonstrated recovery from the reperfusion injury.

To avoid the requirement for bilateral thoracotomies, we attempted initially to perform bilateral lung transplantation in recipient dogs by means of a clamshell incision, which is employed clinically for double-lung transplantation.[9] All six recipients thus approached had shock and died before the transplants could be completed. Presumably, canine car-

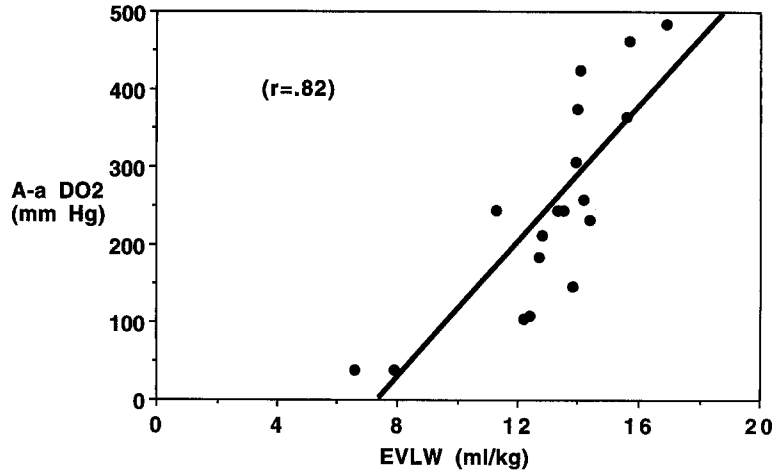

Fig. 5. Scattergram of mean extravascular lung water *(EVLW)* versus mean A-aDo_2 for each interval in both groups of recipients.

diac and venous anatomy does not allow for this surgical approach, which is highly effective in human beings.

In this model, we chose to administer heparin to donors before sacrificing them; in the clinical setting, the same effect might be achieved by the postmortem injection of heparin into the right ventricle, followed by a brief period of cardiopulmonary resuscitation. There is probably a substantial time window for heparin administration; previous studies from our laboratory demonstrated excellent lung function in recipients of nonheparinized lungs retrieved 1 hour after death from nonventilated cadavers.[1] Another potential criticism of this model is that the mode of donor death was controlled. This control was necessary to demonstrate feasibility of cadaveric lung retrieval. In the clinical setting, the mode of death of a potential cadaveric lung donor clearly may set the stage for pulmonary microvascular injury. It would be ideal if a reliable method of postexplantation pulmonary assessment could be developed to predict lung function *after* transplantation.

This study showed that there was no difference in survival or physiologic behavior of recipients between lungs retrieved from cadavers 2 or 4 hours after death. What is the potential time limit for lung retrieval from cadavers? To address this issue, we have studied the time course of pulmonary parenchymal cell death and ultrastructural cell damage after circulatory arrest in the rat. In nonventilated or nitrogen-ventilated rats, with trypan blue exclusion used as a marker of cell viability, 35% of pulmonary parenchymal cells were nonviable 2 hours after death. By 4 hours, this percentage had increased to more than 50%.[10] In contrast, lungs retrieved from cadaveric rats ventilated with oxygen showed only 10% of the cells to be nonviable 4 hours after circulatory arrest and death of the animal. By 12 hours postmortem, only 25% of the lung parenchymal cells in oxygen-ventilated rat lungs were nonviable. Prolonged viability in oxygen-ventilated nonperfused lungs was associated with attenuation of cell autolysis, as assessed by electron microscopy.[11] In short, oxygen ventilation of nonperfused cadaveric lungs appears to delay a substantial fraction of pulmonary cell death beyond 4 hours. Our study supports the notion that lungs retrieved 4 hours after death may be suitable for transplantation; further studies are necessary to determine the maximum allowable time limit, but 4 hours offers some hope of making cadaveric lung retrieval practical in the clinical setting.

It would be ideal to develop a noninvasive method of assessing lung injury that would reliably predict posttransplantation function of lungs retrieved from cadavers. Our observation in these experiments that extravascular lung water was related to A-aDo_2 may be an important fact in the development of noninvasive lung assessment. Recently, we demonstrated a correlation between cell viability and lung tissue levels of adenosine triphosphate and total adenine nucleotides in rat lungs retrieved at varying intervals after death.[12] Both lung water and adenine nucleo-

tide tissue levels can be measured with nuclear magnetic resonance spectroscopy. We are currently investigating the hypothesis that nuclear magnetic resonance evaluation of lung tissue may be useful for predicting the utility of cadaveric lungs retrieved for transplantation.

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